Development of a Recombinase Polymerase Amplification, Lateral Flow Assay to Detect Angiostrongylus cantonensis in Slug Tissue

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Angiostrongylus cantonensis (Rat Lungworm) is a parasitic nematode known to infect humans through ingestion of the third stage larvae and can cause inflammation and damage to the central nervous system. Rat Lungworm disease is the leading cause of eosinophilic meningitis (inflammation of meninges) globally and is becoming increasingly prevalent in the United States and other countries. Currently, quantitative PCR is one of the most reliable diagnostic tests for detecting A.cantonensis, but requires expensive equipment and is time-consuming. This project aims to convert a previously designed recombinase polymerase amplification (RPA) assay into a new isothermal, paper-based lateral flow assay and to compare its sensitivity and accuracy to traditional quantitative PCR. Lateral flow uses inexpensive equipment and quicker setup which can be adapted for use in the field. Slug DNA samples were tested with qPCR, RPA, and lateral flow for A.cantonensis DNA. Eight 10-fold serial dilutions were created using a plasmid containing the target DNA sequence for each assay, ranging from 100 plasmids per µL to 0.78 plasmids per µL. qPCR, RPA, and lateral flow sensitivity were tested using these serial dilutions. Quantitative PCR was able to detect 12.5 plasmids per reaction in each of the three replicates. RPA was able to detect 25 plasmids per reaction in each of the three replicates. Lateral flow was able to detect 50 plasmids per reaction in each of the three replicates. RPA and lateral flow were not as sensitive as qPCR, but were accurate with higher A.cantonensis DNA concentrations. Lateral flow may become a useful alternative to qPCR with further development and has numerous advantages that could make Rat Lungworm diagnostics quicker, less expensive, and more portable.